

NEMATODE POPULATION FLUCTUATIONS IN PINE LITTER AFTER TREATMENT WITH pH CHANGING COMPOUNDS (*)

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Summary

Pine litter treated with a considerable dose of potassium hydroxide results in a very fast increase of the saprophagous nematode population. Other pH increasing products like calcium hydroxide and calcium citrate give the same result but are less pronounced. The effects are of limited duration.

Introduction

The literature treating the effects on the nematode population of the pH and of products that change the pH is scanty and conflicting.

The reason is to be sought in the fact that the pH of agriculture and forest soils is connected with the soil texture and maintained at an ideal level, so that experiments with largely differing pH's did give only poor practical results.

In horticulture, especially in the culture of ornamental plants, there is an increase in the use of substrates which contain, beside a mineral fraction, often a very high percentage of organic or synthetic material.

In such substrates all kinds of interventions are sometimes necessary to optimise the substrate to plant growth. One of these possible interventions consists in changing the degree of acidity. To study the influence of pH on nematodes acids, bases and salts were added to a pine litter soil. The nematodes found are predominantly saprophagous.

Literature

Most data on the influence on nematodes of the pH deal with parasitic nematodes, especially *Heterodera*, *Meloidogyne*, *Pratylenchus* and *Ditylenchus*. The majority of the data relate to salt toleration. (MACHMER (1958), KIRKPATRICK et al. (1959,

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1964), WALLACE (1963), KATZNELSON & HENDERSON (1963), MOUNTAIN (1965), ORTUNA MARTINEZ et al. (1966), BASSUS (1967), HEALD & BURTON (1968), SAALBACH (1968), WALKER (1969, 1971), SIVAPALAN (1972), SASSER (1972), KRYLOV & SHUBINA (1976), SHUBINA (1978), BARANOVSKAJA et al. (1978) and UPADHYAY et al. (1979)).

From these it can be concluded that nematodes can easily stand increasing salt concentrations or the related osmotic pressure, plants on the contrary do eventually suffer.

Changes in the nematode population, however, can appear.

MARSHALL (1977) summarizes them as follows: "Most fertilizers tend to increase total nematode population, but saprophagous and plant parasitic species behave differently". According to literature, exceptions to this general rule are numerous, but the used experimental methods and the circumstances were often very different.

Many data from the earlier literature concern the influence of the pH and inorganic ions on the hatching of these organisms (ELLENBY (1946), FENWICK (1951), ELLENBY & GILBERT (1958), DROPKIN et al. (1958), ROBINSON & NEAL (1959), MARKS & SAYRE (1964), CLARKE & SHEPHERD (1966) and LEHMAN et al. (1971)).

According to STEINER (1952) the pH may be unimportant in nematode ecology and according to WALLACE (1963) evidence of the influence of the pH on plant nematodes is contradictory and further research needed.

BASSUS (1960) found that by adding CaCO_3 or CaO to pine humus the free living nematode population increased by 25% to 100%. According to the latter author and FRANZ (1959) lime never causes a decrease of the nematode population.

On the other hand KUIPER & DE LEEUW (1963) showed that lime dosages increasing the soil pH beyond 7.4 had a marked nematocidal effect on some plant parasitic species.

MORGAN & MACLEAN (1968) also found a marked nematocidal effect with calcium hydroxide on *Pratylenchus penetrans* if the pH surpasses 6.6. Above 7.5 the nematocidal effect was expressed in a rapid decline of the population, the recovered nematodes constituting 17% of the original number. BREZSKI & DOWE (1969) found increasing numbers of *Tylenchorhynchus dubius* when the pH decreased from 7.0 to 4.0.

With higher pH values, up to 8.0, BURNS (1971) recovered more non-stylet nematodes. The *Dorylaimoidea* population was highest at both pH 6 and 8 and for *Pratylenchus alleni* it was highest at pH 6.0. The pH was adjusted by means of sulfuric acid and calciumhydroxide.

It seems that when the host plant has a decreased potassium content there is an increase of the parasitic nematode numbers or of the damage (TARJAN (1950), KIRKPATRICK et al. (1959, 1964), OTEIFA et al. (1964), SASSER (1972), SIVAPALAN (1972)). But WILLIS (1976) could not prove such a straight rule.

OTEIFA & DIAB (1961) suggest that nematodes are associated in some manner with the available potassium of the host. When plants are deficient in potassium nematode damage is increased even if they have a low rate of reproduction. On the other hand when surplus amounts of potassium are used, nematode damage is markedly reduced, even though the rate of reproduction is high. In their opinion the action of fertilizers on the nematode population might only be of significance for about 2 months.

Materials and methods

Experiment I.

At the beginning of December pine litter (*Pinus sylvestris*) was put into plastic containers (10 l substrate per container). The pH varied between 4.2 and 4.7 with a mean of 4.41 and a standard deviation of 0.15 (n=8).

This substrate was treated with acids, bases and both (=salts). We obtained seven modalities with the following doses :

- 1) control
- 2) citric acid (6 g/l substrate)
- 3) nitric acid (6.5 ml HNO₃ 1N per l)
- 4) potassium nitrate (3 g/l)
- 5) calcium citrate (1.6 g Ca(OH)₂ + 4 g citric acid per l)
- 6) potassium hydroxide (3 g/l)
- 7) calcium hydroxide (4 g/l).

The test was carried out with four replicates of 10 l substrate over the following temperature ranges : Dec. 4°-9°C, Jan. 2°-8°C, Febr. 2°-12°C, March 6°-16°C, April 5°-18°C and May 12°-21°C. The humidity was adjusted weekly by means of rainwater. The progress of pH and conductivity (Table I) was achieved by the method of HEUNGENS et al. (1975).

Table I.

Progress of pH and conductivity (μS/cm) in the testsub-
strates (*)

After		1/2 month	1	1 ¹ / ₂	2	3	4 ¹ / ₂	6 months
control	pH	4.4	4.4	4.4	4.3	4.1	4.4	4.5
	μS/cm	95	95	95	120	185	160	200
nitric acid	pH	3.4	3.4	3.4	3.6	3.6	3.7	3.8
	μS/cm	350	375	380	355	370	360	410
citric acid	pH	3.3	3.6	3.9	3.9	3.8	4.2	4.3
	μS/cm	250	175	120	145	145	150	155
potassium- nitrate	pH	3.9	4.0	4.0	4.1	4.1	4.2	4.4
	μS/cm	1035	1030	990	1080	1050	1055	1200
calcium- citrate	pH	5.7	5.6	5.6	5.2	5.0	4.9	4.8
	μS/cm	185	145	90	170	250	275	350
calcium- hydroxide	pH	6.9	7.0	7.0	6.6	6.6	6.4	6.7
	μS/cm	470	360	235	355	475	335	340
potassium- hydroxide	pH	7.3	7.1	7.0	6.7	6.3	6.4	5.6
	μS/cm	560	430	500	570	680	580	950

(*) mean of 4 observations.

Table I shows that pH and conductivity changed during the experiment, due, among others, to enzymatic (bacterial) reversals or carbonate formation of some of the products. The nematodes were collected after 2, 4 and 6 months by taking 10 ml substrate from each modality and container and laid on a wadding filtre on a sieve in a Baermann funnel and drained after 3 days. A heating lamp was not used. The 10 ml were measured by weighing first a litre of substrate and thereafter taking 1/100 of this weight. After draining, the nematodes were concentrated in 20 ml liquid and the number in 2 ml was counted. Thus the number in 1 ml of substrate was obtained.

Experiment II

A substrate composed of *Pinus sylvestris* and *Pinus nigra* needles together with a small quantity of decomposed *Quercus robur* leaves was used as a test substrate. Twenty four plastic bags were each filled with 1 litre of this substrate to which were added 200 ml potassium hydroxide solutions. The KOH-doses per 200 ml distilled water were : 0 - 1/2 - 1 - 2 - 3 and 4 g.

Thus we obtained KOH concentrations of 0 to 4 grammes per litre substrate. After application the substrates had a high water content, the average weight of a litre being 545 g with a standard deviation of 31 g (n=24).

When the bags of substrate reached 450 g, distilled water was added until they weighed 500 g. The test was carried out half March at room temperature (18°-20°C). After two days, 1, 2, 3 and 4 weeks samples of 10 ml were removed and the nematodes extracted and counted.

The levels of acidity and conductivity of the substrates during this test are given in Table II.

Table II.

Acidity (pH) and conductivity (μS/cm) of the substrates during the experiment.

g KOH per 1 substrate		After 2 days	After 1 week	After 2 weeks	After 3 weeks	After 4 weeks
0	pH	4.2	4.6	4.6	4.6	4.8
	μS/cm	40	50	60	70	60
1/2	pH	5.5	5.2	5.3	5.4	5.4
	μS/cm	80	85	90	100	85
1	pH	5.9	5.6	5.8	5.8	5.7
	μS/cm	140	135	160	150	160
2	pH	6.6	6.6	6.8	6.6	6.3
	μS/cm	360	370	320	320	350
3	pH	7.3	7.0	7.2	7.1	6.6
	μS/cm	600	520	450	510	590
4	pH	7.5	7.4	7.8	7.5	7.5
	μS/cm	840	770	640	620	690

Results

Experiment I

The nematodes were saprophagous. The identification of the species, will be carried out later by Dr. P.A.A. Loof (Wageningen). Results of the first experiment are given in Table III, which shows that the substrates treated with acids and the acid working potassium nitrate had a nematode population about half the size of the control population.

Table III.

Average number (\bar{x}) and standard deviation (s) of the total number of nematodes per ml substrate.

After	2 months		4 months		6 months	
	\bar{x}	s	\bar{x}	s	\bar{x}	s
Nitric acid	13.4	3.1	25.5	10.2	11.8	5.8
Citric acid	10.6	2.6	26.5	15.8	40.5	9.4
Potassium nitrate	13.0	2.2	11.0	3.9	21.0	8.8
Control	27.5	3.6	41.8	12.9	38.0	10.9
Calciumcitrate	79.0	23.8	59.3	33.5	56.5	25.2
Calciumhydroxide	73.0	7.1	79.5	40.1	71.3	24.7
Potassium hydroxide	342.8	79.6	98.0	36.5	90.3	24.8
(s) n=8 (4 blocs x 2 repetitions)						

Calcium citrate and calcium hydroxide doubled the population but a more pronounced increase of the population (12x) was obtained with potassium hydroxide.

Relying on an extensive study of the literature we had not expected this latter result. The allegation by OTEIFA & DIAB (1961) that such effects work only during a period of 2 months is remarkable. To permit biometric conclusions, the original data were transformed to logarithms and an analysis of variance carried out, which is summarized in Table IV.

Table IV.

Analysis of variance of the number of nematodes per ml substrate after log-transformation.

Sources of variation	Sum of squares	d.f.	Mean square	F-value	F-table (P=0.01)
Treatments	20.4495	6	3.4083	387.31	3.04
Time	0.0024	2	0.0012	0.14	4.88
Blocs	0.1748	3	0.0583	6.63	4.04
Treat. x Time	4.2340	12	0.3528	40.09	2.41
Treat. x Blocs	0.7627	18	0.0424	4.82	2.18
Time x Blocs	0.6796	6	0.1133	12.88	3.04
Treat. x Time x Blocs	0.9979	36	0.0277	3.15	1.94
Residual error	0.7358	84	0.0088	-	-
Total	28.0367	167	-	-	-

Table IV shows clearly that the treatments had a highly significant influence on the density of the nematode population. Although some of the other sources of variation are also significant, compared to the preceding they are of little importance.

In Table V the summary of the transformed results and also the least significant differences are given.

Table V.

The logarithmic averages of the number of nematodes per ml substrate for the different treatments, the time of sampling and the interaction between both.

After	2 months	4 months	6 months	General log-mean	Retransformed number
Nitric acid	1.116	1.380	1.028	1.175	14.98
Citric acid	1.014	1.358	1.596	1.323	21.04
Potassium nitrate	1.109	1.018	1.293	1.140	13.81
Control	1.434	1.600	1.563	1.532	34.05
Calcium citrate	1.876	1.721	1.719	1.772	59.16
Calcium hydroxide	1.863	1.858	1.826	1.849	70.64
Potassium hydroxide	2.526	1.966	1.940	2.144	139.40
Time-mean	1.563	1.557	1.566		

l.s.d. (p=0.01) for the treatments 0.068
for treatments x time 0.118.

We can conclude that for each treatment significantly different populations were obtained, except for nitric acid and potassium nitrate which are closely similar. The transformed values indicate that the difference is considerable e.g.

139,400/l for potassium hydroxide, 34,500/l for the control and only 13,810/l for potassium nitrate. It is also clear that a pH-effect is present and that the conductivity had no obvious influence (Table I).

The increased population density obtained with KOH after 2 months (336,000 nematodes per litre) cannot be explained by pH alone, but there must be also a typical KOH-effect. This aspect was followed more precisely in experiment II.

Experiment II

The influence of KOH concentration on nematode numbers is given in Table VI.

Table VI.

Average number of nematodes per 10 ml pine litter (4 repetitions).

Grammes KOH per litre substrate	After 2 days	After 1 week	After 2 weeks	After 3 weeks	After 4 weeks
0	361	335	293	270	260
0.5	330	443	363	323	263
1	411	577	708	478	358
2	516	711	2015	943	398
3	258	1870	3258	2935	530
4	208	2253	4965	3005	593

Table VI shows that there exists a clear KOH-influence. However the stimulating effect seems to stop after 3 to 4 weeks. In the control the population density also decreased slightly.

An analysis of variance was carried out for each period separately. To normalise the numbers a $\sqrt[3]{x}$ - transformation appears recommended. The obtained mean and the calculated little significant difference permit us to establish the concentrations between which significant population differences exist.

Table VII.

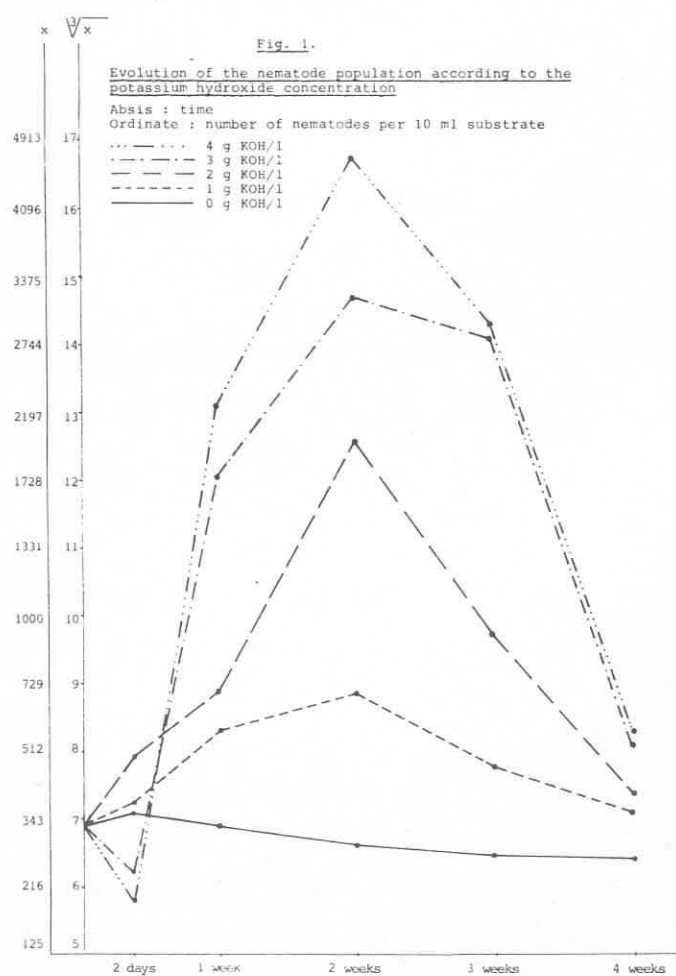
Average number of nematodes per 10 ml (4 repetitions) after $\sqrt[3]{x}$ - transformation.

Grammes KOH per litre substrate	After 2 days	After 1 week	After 2 weeks	After 3 weeks	After 4 weeks
0	7.10	6.87	6.63	6.44	6.38
0.5	6.90	7.48	7.10	6.82	6.38
1	7.23	8.30	8.86	7.77	7.09
2	7.91	8.92	12.56*	9.70*	7.35
3	6.25	12.05*	14.70*	14.11*	8.09*
4	5.81	13.10*	16.75*	14.32*	8.31*
l.s.d. (0.01)	-	2.23	2.67	2.43	1.07

* significant higher number than the control for $p=0.01$.

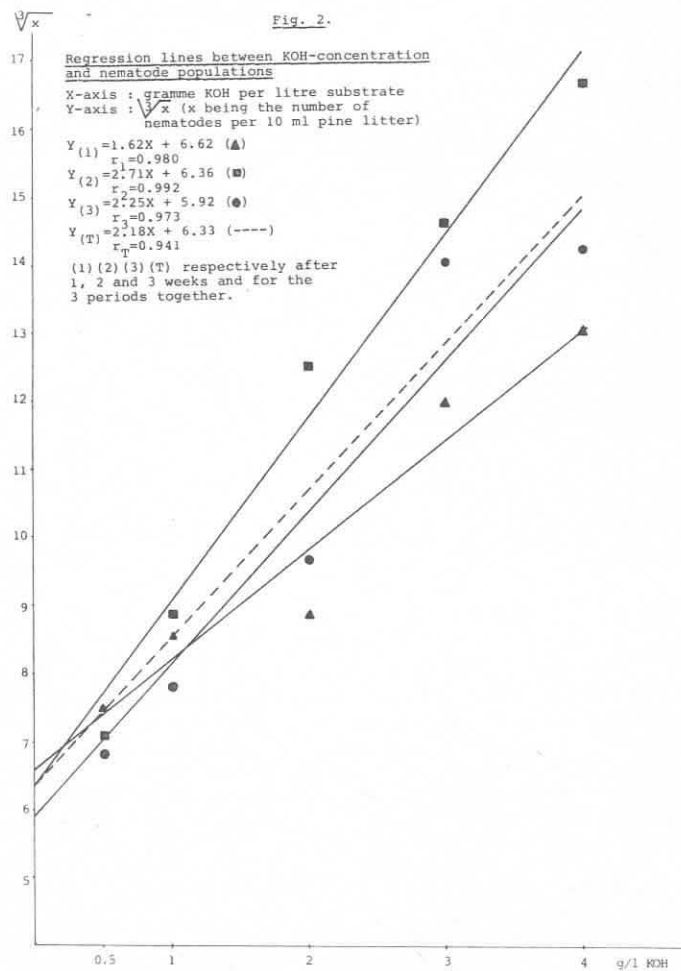
Table VII shows that after 2 days significant population differences were not apparent. A small decrease is occurred at the highest KOH-concentration, it can be described as a shock effect. After 1 week population increases are already visible under the influence of higher KOH doses. They are significant at the doses of 3 and 4 g per litre of substrate. A dose of 2 g KOH/l also has a significant influence after 2 and 3 weeks. After 4 weeks the difference remain significant for the highest doses but are less than those for the preceding week.

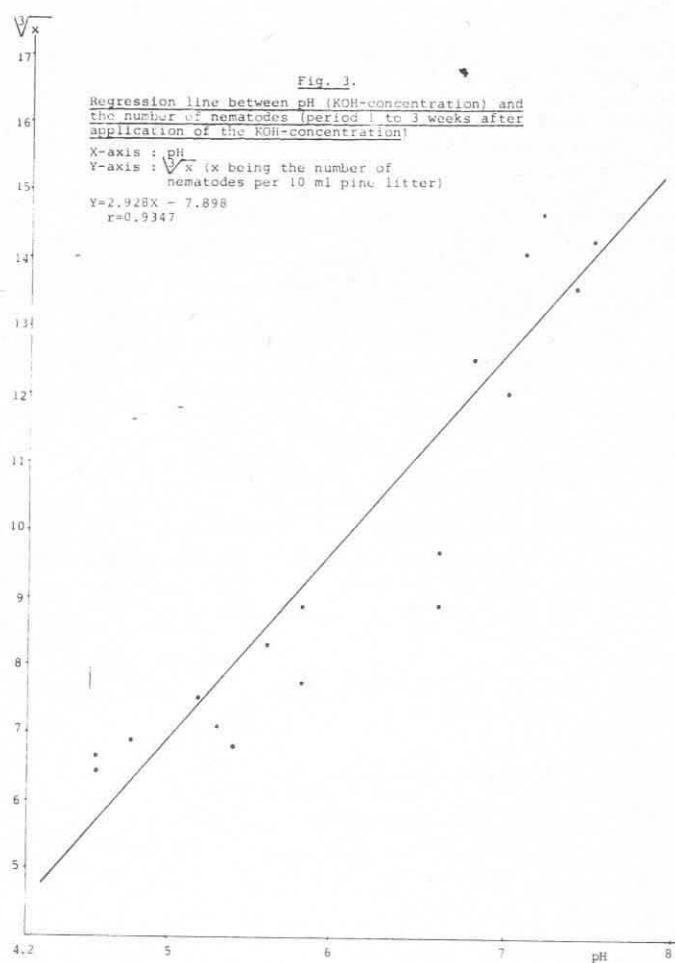
The highest population occurred after 2 weeks. The concentration of 4 g KOH/l gave, after retransformation of the numbers, a population of 470,000 nematodes per litre as compared with 29,100 (or 16 times less) for the control. This population stimulating effect is shown in Fig. 1.



With increasing KOH concentration the nematode population also increases distinctly. The regression of nematode numbers on KOH-concentration was calculated for the periods after 1, 2 and 3 weeks and these 3 periods together from data given in Table VII (Fig. 2).

As the KOH concentration influences pH, a regression line can also be calculated between the pH and nematode numbers. This was done for the period from 1 to 3 weeks (Fig. 3).





Discussion

The first experiment shows that substrates treated with compounds that increase pH contain a higher total nematode population. If nematode numbers are determined principally by pH then differences between the action of calcium citrate and calcium hydroxide should have been much higher and the differences between calcium hydroxide and potassium hydroxide lower. According to the literature studied populations of non parasitic nematodes often increase after treatment with lime and those of parasitic species sometimes decrease. That a rapid increase in numbers occurs after a KOH treatment suggests a faster hatching of the eggs. This could also explain in part the limited duration of the effect. The consulted literature refers clearly to a faster hatching

of eggs after addition of alcalic substances. But this characteristic is attributed also to acids and to some other substances, which means that the concerned mechanism is not yet completely explained. An elevated pH changes the composition of the microbiota in the substrate, with increasing bacterial numbers and less fungi. Saprophagous nematodes feed on bacteria, their increase can thus be explained. It is not clear if the nematode population can follow so quickly the increase of the bacteria. Only the combination of the factor hatching with the factor abundance of food seems at this moment to provide an acceptable hypothesis. Should a potassium or calcium hydroxide dose markedly stimulate the hatching of nematode eggs, then it may be possible to obtain better control results in some cases of nematode attacks. Thus, nematicides applied shortly after such a treatment might give a more complete control. Given the existence of some antagonisms between saprophagous and parasitic organisms and the fact that, their comportment to the discussed products appears to be sometimes completely different depending on their biology, it is possible that controlled pH increases could favor very desirable interactions between the parasitic and saprophagous nematodes and the cultivated plant.

Resumé

Fluctuations des populations de nématodes en litière de pin après traitement aux produits changeant le pH.

Le traitement de litière de pin avec une dose considérable d'hydroxide de potasse résulte en une augmentation élevée de la population des nematodes saprophages. Autres produits qui rehaussent le pH comme l'hydroxide de calcium ou le citrate de calcium donnent le même résultat mais moins prononcé. Les effets sont d'une durée limitée.

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